# Hazardous Virus Detection and Reporting in Real Time

## Edgewood Chemical Biological Center

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# Outline

- Features
- Calibration
- Examples
- Current findings/implications
- Patents
- Reports

# Features:

**Present IVDS System:**

- **Sample in liquid**
- **Weighs - 75 lbs.**
- **Requires operator**

**Improved IVDS System:**

- **Liquid and Air**
- **Weight - 10 lbs.**
- **Automatic operation**
- **No reagents - no chemistry**
- **Rapid results - under a minute**
- **Easy to use - no special skills**
- **Physical Based Counting system for the analysis of all virus and virus like particles - Diagnostics, Domestic Protection, Military Detection**

# Comparing other technologies

| Features | IVDS | PCR | Antibody | BioAssay | Density Gradient | Microscopy – SEM/EM |
|---|---|---|---|---|---|---|
| Easy to Operate | **Yes** | No | No | No | No | No |
| Few Supplies | **Yes** | No | No | No | No | No |
| Fast Answers | **Yes** | No | No | No | No | No |
| Gives Concentration | **Yes** | No | No | Partial | No | Yes |
| Gives Size | **Yes** | No | No | No | No | Yes |
| Multiple viruses at same time | **Yes** | No | No | No | Yes | Yes |
| Identify families | **Yes** | Yes | Yes | Yes | No | Yes |
| Detects Unknowns | **Yes** | No | No | Partial | Yes | Yes |
| Simple to use | **Yes** | No | No | No | No | No |
| Low Operating costs | **Yes** | No | No | No | No | No |
| Auto save to Disk | **Yes** | No | No | No | No | No |
| Separates viruses from background | **Yes** | No | No | No | No | No |
| Operate Unattended | **Yes*** | No | No | No | No | No |
| Act as a Detector | **Yes*** | No | No | No | No | No |
| Sensitive to low levels | **Yes*** | No | No | Yes | No | Yes |

- VDCS-3

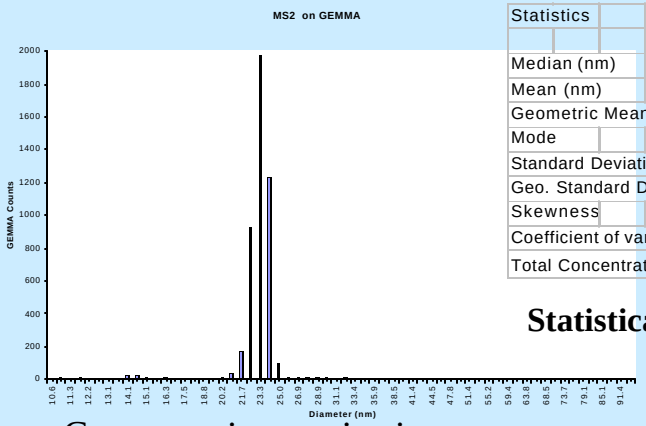
# Calibration

**Calibrated by National Institute of Standards & Technology (NIST)**

* Particle Size
* Particle Concentration

Used NIST standards, used commercial standards, and used new standards developed in cooperation with NIST

## Integrated Virus Detection System (IVDS) data




Concentration vs size in nanometers

| Statistics | |
|---|---|
| Median (nm) | 23.3 |
| Mean (nm) | 23.1 |
| Geometric Mean (nm) | 22.9 |
| Mode | 23.3 |
| Standard Deviation | 3.1 |
| Geo. Standard Deviation | 1.1 |
| Skewness | -0.1 |
| Coefficient of variation (%) | 13.6 |
| Total Concentration (#/ml) | $1.13 \times 10^5$ |

**Statistical information**

Virus analysis by IVDS:

- Actual count data
- Statistical data
- Distribution table.

Notice that both the size and concentration are clearly demonstrated.

**Table with size distribution data**

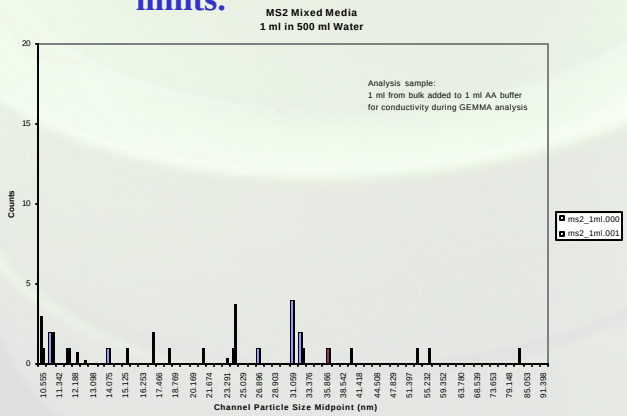
| Diameters | Counts |
|---|---|
| 10.6 | 4 |
| 10.9 | 6.7 |
| 11.3 | 5.3 |
| 11.8 | 7.9 |
| 12.2 | 4.1 |
| 12.6 | 3.1 |
| 13.1 | 3.9 |
| 13.6 | 3.8 |
| 14.1 | 28.9 |
| 14.6 | 23.7 |
| 15.1 | 7.5 |
| 15.7 | 6.1 |
| 16.3 | 9 |
| 16.8 | 3 |
| 17.5 | 3 |
| 18.1 | 3 |
| 18.8 | 6.5 |
| 19.5 | 5.5 |
| 20.2 | 14.3 |
| 20.9 | 40.3 |
| 21.7 | 175.1 |
| 22.5 | 930.9 |
| 23.3 | 1968 |
| 24.1 | 1239 |
| 25.0 | 105.7 |
| 25.9 | 18.9 |
| 26.9 | 12.8 |
| 27.9 | 10.5 |
| 28.9 | 8.4 |
| 30.0 | 9.4 |
| 31.1 | 5.6 |
| 32.2 | 12 |
| 33.4 | 5 |
| 34.6 | 4 |
| 35.9 | 4 |
| 37.2 | 2 |
| 38.5 | 1 |
| 40.0 | 2 |
| 41.4 | 2 |
| 42.9 | 3 |
| 44.5 | 0 |
| 46.1 | 4.3 |
| 47.8 | 4.7 |
| 49.6 | 1 |
| 51.4 | 1 |
| 53.3 | 1 |
| 55.2 | 3 |
| 57.3 | 3 |
| 59.4 | 5 |
| 61.5 | 4 |
| 63.8 | 0 |
| 66.1 | 1 |
| 68.5 | 0.6 |
| 71.0 | 0.4 |
| 73.7 | 0 |
| 76.4 | 0 |
| 79.1 | 0 |
| 82.0 | 1 |
| 85.1 | 0 |
| 88.2 | 0 |
| 91.4 | 1 |
| 94.7 | 0 |

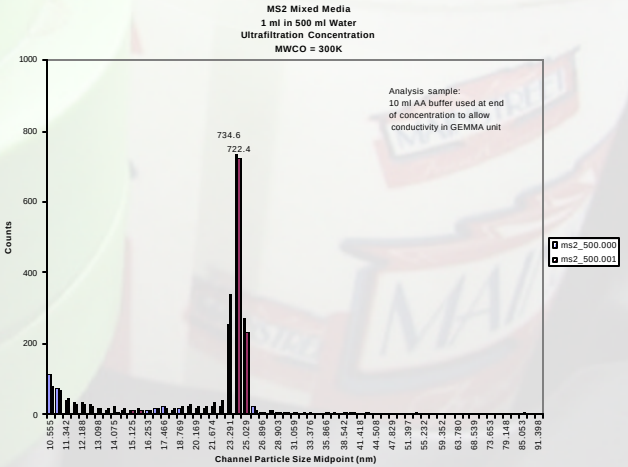
# Drinking Water samples

**Sample illustrates MS2 maker used as an indicator virus.**

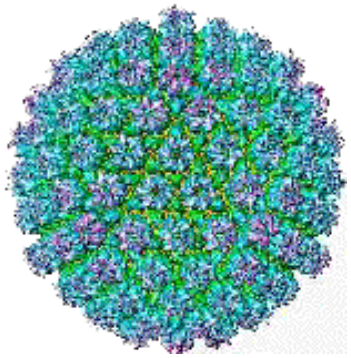
* **500 ml water sample processed through UF to final volume of 1.2 ml.**
* **300 K MWCO filter used.**
* **750 viruses counted.**

* **500ml of water with MS2 below detection limits.**



# VIRUS CONCENTRATION AND SIZE ARE BOTH IMPORTANT FOR DETERMINING STATUS OF PATHOGENS



VIRUS SIZE PROVIDES IDENTITY OF VIRUSES

- FOOT AND MOUTH VIRUS – 30 NM
- YELLOW FEVER VIRUS – 45 NM
- VEE/WEE VIRUSES – 70 NM
- INFLUENZA – 100 NM

VIRUS CONCENTRATION PROVIDES CONDITION AND TRENDS:

- DOWN WIND HAZARD PREDICTION
- HOW MUCH OF A THREAT IS PRESENT
- MAY PROVIDE INFORMATION ON THREAT DURATION

# Hazardous Virus Detection and Reporting in Real Time

## Edgewood Chemical Biological Center

### CHARACTERIZATION OF PURIFIEI BACTERIOPHAGE BY THE PHYSICAL C METHODOLOGY USED IN THE INTEGRA DETECTION SYSTEM (IVDS)

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*A new physically based methodology—the integrated vir (IVDS)—was used to characterize a high-concentration, sample preparation of MS2 bacteriophage with a reported units (pfu)/mL (DPM14) virus count in a common TNME were made using the IVDS instrument following serial dicated virus counts of $1.5 \times 10^5$ for the neat sample (D $6.5 \times 10^4$ viruses (DPM13), $1.2 \times 10^4$ viruses (DPM12), $9.3 \times 1$ 88 viruses (DPM10), and 5 viruses (DPM9), respectively. displayed a consistent multiplier and were consistent wi Increases in virus concentration appear to decrease the through aggregation. The results demonstrate a consisten methodology. The results further indicate that the IVDS ins for characterization of other virus preparations with equ results.*

**Keywords** Bacteriophage, virus counting, virus analys Detection System, IVSD, virus detection

The detection and analysis of viruses has been the go many years, following the first evidence that a new typ ism was responsible for diseases in both humans and viruses were smaller than bacteria and thus presente lenge. Their small size made classification of these ne cult and the field of virology was advanced by biochen rather than by direct examination. Advancements in ele have helped solve this problem and much information h on the physical features of more than 21 virus familie techniques are, however, time-consuming, and requir edge, chemicals or reagents, and techniques to be succ

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### PASSAGE OF MS2 BACTERIOPHAGE THROUGH VARIOUS MOLECULAR WEIGHT FILTERS

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*MS2 bacteriophage has a reported nominal molecular weight of 2M daltons. It would be expected that this phage would not pass through filters of various sizes with low molecular weight cutoff (MWCO) values of less than 1M daltons. It was discovered that MS2 bacteriophage will pass through filters with 750K-, 500K-, and 300K-dalton MWCO values. MS2 was retained on the 100K-dalton filter. A cross-flow hollow fiber apparatus was used for the 750K- and 500K-dalton analysis. Centrifuge filters of 1M and 300K and 100K daltons were used. The rate of passage of MS2 through the cross-flow filters is dependent on the tangential flow rate and pressure. Passage through the centrifuge filters depended on the gravitational force applied.*

**Keywords**

Nominal molecular weight cutoff (MWCO) values of various filters can lead to the assumption that items larger than the cutoff values will be retained after filtration. However, at least for MS2 bacteriophage, there are exceptions. It was discovered during the operation of the integrated virus detection system (IVDS) instrument that counts of MS2 decreased during ultrafiltration and purification, and for the detection of small numbers of viruses, any loss is important. This observation led to further investigation.

This study was initiated to better understand the filtration characteristics of MS2 bacteriophage. Different filtration techniques and their relative filtration effectiveness were explored. The sample of MS2 bacteriophage, used in the filtration studies, was received from the Life Sciences Division at Dugway Proving Ground (DPG). This sample was 2 mL of purified MS2 bacteriophage at a concentration $1 \times 10^{14}$ plaque-forming units (pfu)/mL or 10.2 mg protein/mL. This highly purified sample was from lot #98110.

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### PURIFICATION OF MS2 BACTERIOPHAGE FROM COMPLEX GROWTH MEDIA AND RESULTING NALYSIS BY THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

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*Purification and concentration of viruses from the background material is required whatever subsequent analysis methods are used. For the analysis of viruses it is essential and detection methods depend on this solution. This report demonstrates a methodology for the removal of growth media from a virus preparation. A sample of MS2 was purified using a new ultrafiltration (UF) technique with hollow fibers. A typical MS2 virus sample with a nominal stated concentration of $1.4 \times 10^{12}$ plaque-forming units (pfu)/mL in the original growth media was used to demonstrate this method. After UF, the growth media was removed and the virus counted using the integrated virus detection system (IVDS) instrument. This report further describes the use of this ultrafiltration procedure to remove other impurities, such as cesium chloride and albumin, from solutions containing a purified solution of MS2 bacteriophage. These solutions were also analyzed using the IVDS instrument.*

**Keywords** Bacteriophage, virus counting, virus analysis, Integrated Virus Detection System, IVDS, virus filtration, virus ultrafiltration

re are many inherent challenges to virus detection and analysis. of the more important is purification and concentration from the ground material. This is required whatever the detection method to sed in subsequent steps. The background loading, which may contain vth media, salts, proteins, and other material, all make this issue a llenge. It is possible that there is little purpose in even considering ction of viruses until these steps have been taken. One step is the oval of growth media and other impurities such as salts and proteins. A sample of MS2 bacteriophage was received from the Life Sciences ision at Dugway Proving Ground (DPG). This sample was 500 mL of rown MS2 bacteriophage, complete with growth media, at a virus centration of $1.4 \times 10^{12}$ plaque-forming units (pfu)/mL. The growth

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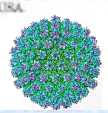
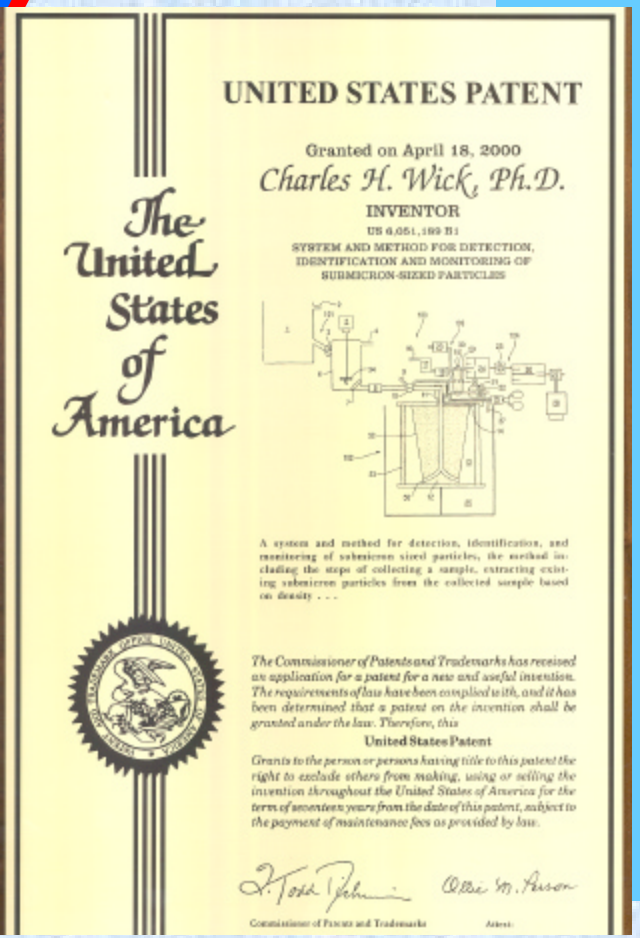


# Current Findings:

- Virus sampling from the environment (need to calibrate samplers).
- Unexpected virus survival following harsh conditions (may provide information on threat duration).
- Unexpected intact Virus components following harsh physical conditions (may impact thinking on other types of detection).
- Unexpected passage through filter media in a liquid environment (need to examine filter media/virus relationships).

**Patents**:


UNITED STATES PATENT

Granted on April 18, 2000

Charles H. Wick, Ph.D.

INVENTOR

US 6,051,189 B1

SYSTEM AND METHOD FOR DETECTION, IDENTIFICATION AND MONITORING OF SUBMICRON-SIZED PARTICLES

The United States of America

A system and method for detection, identification, and monitoring of submicron sized particles, the method including the steps of collecting a sample, extracting existing submicron particles from the collected sample based on density . . .

The Commissioner of Patents and Trademarks has received an application for a patent for a new and useful invention. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law. Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

1. C. H. Wick, with D. Anderson, *System and Method for Detection, Identification and Monitoring of Sub-micron Sized Particles*. Patent number 6,051,189 issued April 18, 2000.
2. C. Wick *Method & System for Separating & Counting Submicron Sized Particle Aerosols*.- filed.
3. C. Wick *Method & System for Detecting & Recording Submicron Sized Particles* - filed.
4. C. Wick *Method & Apparatus for Calibrating and Counting Submicron Sized Particles* – filed
5. Others pending

- **Patent License Agreement**: ECBC awarded an exclusive license to Virus Detection Systems Company, L.L.C., Solomons, MD for the commercial rights to this Integrated Virus Detection System (IVDS) technology. The IVDS technology has been assessed to have wide national and international commercial markets.
- **2002 Award for Excellence in Technology Transfer**, by the Federal Laboratory Consortium for Technology Transfer.

- C.H. Wick, McCubbin, P.E., *Filtration Characteristics of MS2 Bacteriophage Using Various Molecular Weight Filters*, ECBC-TR-54, August 1999

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• Wick, C.H., McCubbin, P.E., *Purification of MS2 Bacteriophage from Complex Growth Media and Resulting Analysis by the Integrated Virus Detection System (IVDS).* Toxicology Methods, 9: 253-263, 1999.

# Point of Contact

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